

Development of a Sample Preparation Technique for Supercritical Fluid Extraction for Multiresidue Analysis of Pesticides in Produce

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Supercritical fluid extraction (SFE) of fruits and vegetables poses unique sample preparation considerations because the sample size is small (1–3 g) and the analyte is distributed in a moist solid matrix. The goal of this research was to develop practical sample preparation procedures for SFE of pesticide residues in produce so that acceptable accuracy and precision are maintained. In this study, 130 extractions of potato, fortified with up to 40 pesticides, were performed with 2 commercial SFE instruments. Extracts were analyzed by gas chromatography with ion trap mass spectrometry or electron capture detection. Four sample preparation procedures were tested and Hydromatrix was used to control the amount of water in the sample. The highest recoveries and lowest standard deviations were obtained when 20–50 g samples were blended with an equal amount of Hydromatrix and dry ice was added to keep the samples frozen. The dry ice helped produce a homogeneous flowable powder and greatly reduced the degradation or vaporization of several pesticides. Recoveries of most pesticides from subsamples of <4 g with this procedure were 90–105%, with relative standard deviations of 1–6%. Only diphenylamine and disulfoton gave reduced recoveries with this procedure. When samples were extracted sequentially with an autosampler, certain pesticides were degraded in the extraction vessels over a period of several hours. To avoid losses of these pesticides, the sample in the extraction vessel was either purged with CO₂ to remove oxygen or kept frozen until extracted. Peach and orange check samples were analyzed with the method, and results were comparable with those from traditional analyses.

Supercritical fluid extraction (SFE) is a new technology for extraction of a wide range of chemicals from many sample matrixes (1–4). An increasing number of publications on applications of SFE to analysis of pesticides in foods indicates the strong interest in and potential of this new technique (5–15). SFE is gaining acceptance as an alternative to solvent-based extraction methods but rarely has been applied for routine analysis. SFE offers an environmentally safer extraction; essentially obviates use of organic solvents; generates very little waste; reduces time, space, and glassware required for extraction; and enables automation.

Sampling and sample preparation for residue analysis—so that results are accurate, reproducible, and representative—have been evaluated thoroughly when current methods were being developed (16–18). Conventional sample preparation for multiresidue pesticide analysis involves chopping a frozen sample with a Hobart cutter and extracting subsamples of 50–100 g with organic solvents (19–21). With SFE, it is not practical to use a liquid solvent to disperse analytes in a homogeneous solution before extraction, and a different sample homogenization approach must be developed. Water in the sample must be controlled so that extraction efficiency is not affected. Also, sample size for SFE is usually small (1–3 g plant material); therefore, to obtain a representative subsample of that size, homogenization of the larger sample is required. Moreover, without solvents, the analyte in the solid sample becomes more vulnerable to evaporation and degradation.

The objective of this study was to develop a novel sample preparation approach for SFE of pesticide residues that addresses the unique characteristics of SFE and still maintains the accuracy and precision of the current solvent-based extraction methods.

Experimental

Apparatus

(a) *Supercritical fluid extractors.*—A Model 7680T (Hewlett-Packard, Little Falls, DE) and a Prepmaster (Suprex, Pittsburgh, PA), both equipped with automated variable restrictors and solid sorbent collection systems, were used. The 7680T was automated so that 8 vessels could be loaded into a carousel and extracted in sequence. The Prepmaster was oper-

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ated manually. For the 7680T, extraction parameters were as follows: extraction pressure, 320 atm; temperature, 60°C (CO₂ density, 0.85 g/mL); 7 mL extraction vessel, 2 min static extraction followed by 42 mL CO₂ at a flow rate of 1.6 mL/min; 50°C restrictor temperature; collection on (octadecylsilica) ODS sorbent trap (1 mL) at 9°C; and elution with 1.5 mL acetonitrile at 0.4 mL/min and 50°C. The trap was rinsed to waste with 2 mL ethyl acetate followed by 2 mL acetonitrile at 2 mL/min to clean and regenerate the ODS between extractions. For the Prepmaster, instrument settings were the same except for the following: vessel size, 5 or 10 mL (30 or 60 mL CO₂ extraction volume, respectively); C₁₈ trap material mixed with Unibeads; trap elution at 40°C with 1.6 mL acetonitrile; N₂ gas at 50 psi to blow the trap dry; and 5 mL acetonitrile at 2 mL/min to flush the trap between extractions.

(b) *Gas chromatographs*.—A Model ITS40 gas chromatograph/ion trap mass spectrometer (GC/ITMS; Finnigan MAT, San Jose, CA), consisting of a Varian 3300/3400 gas chromatograph and a CTC A200S autosampler, and a Model 5890 gas chromatograph (Hewlett-Packard) equipped with a Model 7673 Hewlett-Packard autosampler, electron capture detection (ECD), and nitrogen-phosphorus detection (NPD) were used. To analyze a 40-pesticide mixture, the following operating conditions were used: 1 µL injection volume into a Model 1093 (Varian, Walnut Creek, CA) septum-programmable injector; 55°C injection port for 30 s followed by ramping to 250°C at 250°C/min; 6 psig He column head pressure; 55°C initial oven temperature for 30 s, ramped to 130°C at 50°C/min, then to 165°C at 1.5°C/min and to 250°C at 4°C/min, and held at 250°C until a total time of 60 min had elapsed; 240°C transfer line temperature; and 215°C detector manifold temperature. Conditions were the same for GC/ITMS analysis of chlorinated pesticides, except that the oven temperature program was 60°C to 130°C at 50°C/min and then to 250°C at 7.5°C/min and hold for 20 min. Typical ITMS operating conditions were as follows: electron impact mode; 10 µA filament current; 1500 V electron multiplier tube; 1 ms ion time; and automatic gain control at 20 000. The GC-ECD and GC-NPD conditions were: 1 µL splitless injection volume; 250°C injection port; 0.5 min purge delay; 21 psig He column head pressure (2.6 mL/min); 100°C initial oven temperature to 220°C at 3°C/min ramp rate; 300°C ECD temperature; 43 mL/min ECD makeup gas flow rate of 5% CH₄ in Ar; 260°C NPD temperature; 3.5 mL/min H₂, 100 mL/min air, and 30 mL/min He NPD gas flow rates.

(c) *Chromatographic columns*.—A DB-1701 or a DB-5ms (J&W Scientific, Folsom, CA), 30 m, 0.32 mm id, 0.25 µm film thickness capillary column and a 5 m phenylmethyl deactivated (Restek Corp., Bellefonte, PA) guard column (0.32 mm id) were used for GC/ITMS. For GC-ECD, a 100% dimethylpolysiloxane SPB-1 (Supelco, Bellefonte, PA) 30 m, 0.25 mm id, 0.25 µm film thickness capillary column was used, and for GC-NPD, a DB-17 (J&W Scientific) 30 m, 0.32 mm id, 0.25 µm film thickness capillary column was used.

(d) *Data collection*.—For GC/ITMS, a Magnum version 2.4 software package (provided with the instrument) loaded into a Gateway 2000 computer was used. For GC-ECD and GC-NPD, a Pascal version Chemstation software package

loaded into a Hewlett-Packard 300 series computer was used for data collection and analysis and instrument control. For the ion trap, the data collection range was 65–425 *m/z* from 5 to 60 min for analysis of 40 pesticides and from 6 to 20 min for analysis of chlorinated pesticides only.

Reagents

(a) *Gases*.—SFC/SFE grade CO₂ (Air Products, Allentown, PA), with a He headspace of 1800 psi (for Prepmaster) or without He headspace (for 7680T), was used. Bone-dry grade CO₂ (for both instruments) and N₂ (for Prepmaster) were required for cryogenic cooling and drying of the trap, respectively. The septum-programmable injector on the GC/ITMS also used CO₂ with dip-tube for cooling.

(b) *Solvents*.—Acetonitrile and ethyl acetate, pesticide grade (Fisher, Fair Lawn, NJ).

(c) *Solids*.—Hydromatrix (HMX, Varian, Harbor City, CA), a pelletized diatomaceous earth, was washed with acetone before use to remove contaminants. Use of HMX in SFE has been described previously (8). The preppacked 30 µm Hypersil ODS (Hewlett-Packard) traps were provided with the 7680T; for the Prepmaster, 35 µm C₁₈ mixed with Unibeads (Suprex) were contained in the trap.

(d) *Pesticide standards*.—Pesticides were obtained from the U.S. Environmental Protection Agency (Research Park, NC, or Beltsville, MD). Chrysene-d₁₂ (Cambridge Isotope Laboratories, Woburn, MA) was used as internal standard for the 40 pesticides, and pentachloroanisole (PCAS) or aldrin was used as internal standard for the chlorinated pesticides. Individual stock solutions were prepared by weighing 10–12 mg amounts of standards, dissolving the pesticide in acetone and/or isooctane, and making up to 100 mL in volumetric flasks. Concentrations were corrected for the stated purities (typically >98%) of the standards. Working standard mixtures in acetone, containing 20 µg/mL for each pesticide, were used for spiking samples and preparing calibration standards.

Sample Preparation

Comercially purchased potatoes served as blank or fortified samples. An outline of experiments is given in Table 1. These experiments evolved from the need to produce a homogeneous, representative sample without use of liquids. The initial attempt (Experiment 1) was simply to prepare a homogeneous sample by using a Hobart cutter. The second experiment improved homogeneity by mixing a representative portion of the sample with HMX in a blender. In Experiment 3, the sample preparation procedure included 40 pesticides. In the last experiment, the procedure was refined by using dry ice during blending to improve mixing and reduce losses of certain pesticides.

Experiment 1

In this experiment, 19 potatoes (2.3 kg) were cut into quarters, and 19 pieces (25%) were randomly separated and spiked with hexachlorobenzene (HCB) at 0.41 µg/g and lindane at 0.43 µg/g (50 µL of 1000 µg/mL HCB and 184 µL of 286 µg/mL lindane on each piece). The spiking solvent was allowed to evaporate for 1 h, and then all 76 pieces were com-

Table 1. Procedures for sample preparation used in different experiments

Parameter	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Initial sample size	2.3 kg	2.3 kg	50 g	50 g
Spiking level(s)	0.413 µg/g, HCB; 0.434 µg/g, lindane	0.413 µg/g, HCB; 0.434 µg/g, lindane	0.4 µg/g, 37 pesticides	0.2 or 0.4 µg/g, 40 pesticides
Sample processing	Chopped, frozen (100 g subsample); rechopped frozen (20 × 50 g subsamples)	50 g subsample from Experiment 1 blended with 100 g HMX	Blended with 100 g HMX	Blended with 50 g HMX + dry ice
Packing of extraction vessel (vessel volume)				
7680T	2.1 g potato + 1.4 g HMX (7 mL)	1.3 g potato + 2.7 g HMX + 1 mL H ₂ O (7 mL)	1.3 g potato + 2.7 g HMX + 1 mL H ₂ O (7 mL)	2 g potato + 2 g HMX (7 mL)
Prepmaster	3 g potato + 2 g HMX (10 mL)		1 g potato + 2 g HMX + 1 mL H ₂ O (5 mL)	1.5 g potato + 1.5 g HMX (5 mL)
No. of extractions				
7680T	48	9	14	15
Prepmaster	32		6	6
Method of analysis	GC/ITMS, GC-ECD	GC/ITMS, GC-ECD	GC/ITMS	GC/ITMS

bined and shredded with a Handi-Shortcut II food processor (Black & Decker, Shelton, CT). The shredded potatoes were stored overnight at -20°C . On the next day, 100 g sample was separated, and while still frozen, the remaining potato sample was chopped further with a Model 84142 cutter (Hobart, Troy, OH). The potato sample was divided into twenty 50 g portions and stored at -20°C until extraction. For SFE with the 7680T, 8 subsamples of 2.1 g each from the 100 g portion of potato and 4 subsamples (2.1 g each) from 10 of the 50 g portions (48 extractions total) were extracted in the course of 5 days (1.4 g HMX was mixed with each frozen 2.1 g subsample in a beaker with a glass rod before packing into a 7 mL extraction thimble). For SFE with the Prepmaster, three 3 g subsamples from 9 of the 50 g portions were extracted over the course of 3 days (2 g HMX was added to each sample before packing into a 10 mL extraction vessel). In all cases, 15 µL of 20 µg/mL pentachlorobenzene (PCB) was added to the sample in the vessel as an SFE matrix spike before extraction. An internal standard, aldrin or PCAS, was added to extracts at 0.5 µg/mL before analysis by GC/ITMS and GC-ECD.

Experiment 2

A 50 g portion of frozen potato from Experiment 1 was mixed with 100 g HMX in a blender (Waring, New York, NY). The sample became a flowable powder, and because the moisture content of the powder was lower than what had been used in previous studies (5–8), 1 mL water was added to the sample in the vessel before extraction. For SFE with the 7680T, eight 4 g subsamples (1.33 g potato and 2.67 g HMX) were extracted on the same day. As the control spike, 50 µL of 20 µg/mL PCB

was added to the sample in the vessel before extraction. Aldrin was the internal standard for GC/ITMS and GC-ECD.

Experiment 3

Fresh potato (50 g) was sliced into 12 pieces with a knife, and 4 pieces were each spiked with 250 µL of a mixture of 40 pesticides in acetone containing 20 µg of each pesticide per mL (0.4 µg/g fortification level for the 50 g sample). The solvent was allowed to evaporate at room temperature for 1 h. The 12 pieces were blended with 80 g HMX for 5 min into a flowable powder, as in Experiment 2. For SFE with the 7680T, fourteen 4 g subsamples were extracted sequentially over the course of 2 days, and for the Prepmaster, six 3 g subsamples (in 5 mL vessels) were extracted on the same day. A 20 g potato sample blended with 40 g HMX served as a blank and control spiking matrix. Five 4 g subsamples were spiked in the extraction thimbles with 35 µL of the working standard mixture of 40 pesticides, and 4 other subsamples were extracted as blanks. The blank extracts were combined and used to prepare calibration standards for GC/ITMS.

Experiments 4A and 4B

Potato (50 g) was fortified with 20 pesticides at 0.2 µg/g each and the 20 other pesticides at 0.4 µg/g each. The fortified sample was placed in a precooled blender jar with dry ice and 50 g HMX. The sample was blended for 5 min and kept cold by addition of dry ice. In Experiment 4A, a portion of the sample was placed in the freezer and later loaded into 5 vessels and extracted individually; the sample was kept frozen until extracted by SFE. In Experiment 4B, 7 subsamples (4 g each) from the second portion were loaded into extraction vessels and

kept in the 7680T carousel at room temperature until analyzed in sequence. The first 5 samples were analyzed 1 h apart, and the 6th and 7th samples were analyzed 19 and 20 h after the first sample. The 6th thimble was kept in supercritical CO₂ from the 6th to the 19th hour. Experiment 4A was carried out also on the Prepmaster; six 3 g samples (1.5 g potato) in 5 mL thimbles were extracted in this manner.

Check Samples

Peach and orange check samples were provided by the California Department of Food and Agriculture (CDFA) as part of a quality assurance protocol for laboratories participating in the Pesticide Data Program (22). Samples consisted of unknown incurred and/or fortified pesticides at unknown concentrations. The pesticides were not evenly distributed when fortified into the sample by the CDFA. Frozen sample (100 g) was mixed with 100 g HMX and a small amount of dry ice in a blender, as in Experiment 4. Because of the larger sample size, about 25 g sample and HMX were blended at a time before mixing in another portion. In each case, 6 extraction vessels were loaded with 4 g subsamples (2 g sample) of the cold homogenate, and 15 µL of 25 µg/mL aldrin was added to each vessel. Two of the vessels were fortified with 50 µL of the 20 µg/mL working standard mixtures (equivalent to 0.5 µg/g for 40 pesticides in the sample). The capped vessels were purged with CO₂ gas before being loaded in the 7680T carousel. After extraction, 15 µL of 40 µg/mL chrysene-d₁₂ was added to each 1.5 mL extract.

The method of standard additions was used for quantitation of check samples. Three of the 4 extracts were combined, and four 1 mL aliquots were added to autosampler vials with a syringe (the excess served as a second control extract). Microsyringes were used to add 2.5, 7.5, 22.5, and 62.5 µL of the 20 µg/mL pesticide standard mixture to give 0.05, 0.15, 0.45, and 1.35 µg/mL added standards which corresponded to 0.038, 0.112, 0.338, and 1.01 µg/g in the 2 g samples. For peaches, the controls and the 0.05 and 0.15 µg/mL added standards were analyzed twice; for all other cases, controls and standards were analyzed once.

Analysis

(a) *Calibration*.—For GC/ITMS calibration, the spiking solutions were diluted to make the calibration standards. For best quantitation, the calibration standards were prepared in SFE extracts from sample blanks of the same matrix. Calibration standards were 0.1, 0.2, 0.5, and 1.0 µg/mL for analysis of the 40 pesticides in potato. For GC/ITMS analysis of chlorpropham incurred in potato, 5.0, 2.5, 1.0, and 0.5 µg/mL calibration standards were prepared.

(b) *Data analysis*.—Integrated peak area data of selected masses versus the chrysene-d₁₂ internal standard were used for GC/ITMS quantitation. Table 2 lists retention times (*t_r*) and masses chosen for quantitation of each pesticide (*m/z* = 240 amu for chrysene-d₁₂). Quantitation of GC-ECD data was by integrated peak area of selected masses versus the aldrin or PCAS internal standard. Calculations were done with a spreadsheet program or the instrument's software program. For fur-

Table 2. Retention times (*t_r*) of pesticides with the DB-5ms column and quantitation masses for GC/ITMS analysis

No.	Pesticide	<i>t_r</i> , min	Masses ^a , <i>m/z</i>	No.	Pesticide	<i>t_r</i> , min	Masses ^a , <i>m/z</i>
1	Dichlorvos	4.5	109 ⁺ + 127 + 185	21	Vinclozolin	25.5	198 + 212 ⁺ + 285
2	α-Mevinphos	7.4	127 ⁺ + 164 + 192	22	Carbaryl	26.0	115 + 116 + 144 ⁺
3	PCB ^b	9.5	248 + 250 ⁺ + 252	23	Malathion	29.4	125 + 127 + 173 ⁺
4	Diphenylamine	13.1	167 + 168 + 169 ⁺	24	Chlorpyrifos	29.4	197 + 199 + 314
5	Ethoprop	13.6	97 + 158 ⁺ + 243	25	Dacthal	29.5	299 + 301 ⁺ + 303
6	Chlorpropham	14.6	127 ⁺ + 171 + 213	26	Parathion	30.0	97 + 109 ⁺ + 291
7	HCB ^c	15.8	282 + 284 ⁺ + 286	27	Methidathion	33.7	85 + 93 + 145
8	Phorate	15.9	75 ⁺ + 121 + 260	28	Endosulfan I	34.1	195 + 241 ⁺ + 339
9	Dicloran	17.0	124 ⁺ + 176 + 206	29	DDE	36.0	246 + 316 ⁺ + 318
10	Dimethoate	17.3	87 ⁺ + 93 + 125	30	DDT	38.5	165 + 235 ⁺ + 237
11	PCNB ^d	18.1	295 + 297 + 299	31	Ethion	38.6	97 + 153 + 231 ⁺
12	Carbofuran	18.2	149 + 164 ⁺	32	Propargite	41.6	135 ⁺ + 173 + 350
13	Lindane	18.6	181 ⁺ + 183 + 219	33	Phosmet	42.8	160 ⁺
14	Atrazine	18.7	200 ⁺ + 215 + 216	34	Iprodione	42.9	314 ⁺ + 316
15	Terbufos	19.7	231 ⁺	35	Methoxychlor	43.5	227 ⁺
16	Chlorothalonil	20.1	264 + 266 ⁺ + 268	36	Phosalone	44.7	182 ⁺ + 184 + 367
17	Diazinon	21.0	137 + 179 ⁺ + 304	37	Azinphos-methyl	44.8	132 ⁺ + 160
18	Disulfoton	21.3	88 ⁺ + 89 + 97	38	cis-Permethrin	48.2	127 + 163 + 183 ⁺
19	Phosphamidon	24.6	72 + 127 ⁺ + 264	39	Fenvalerate	55.4	125 + 225 ⁺ + 419
20	Parathion-methyl	25.4	109 + 125 + 263 ⁺	40	Esfenvalerate	56.6	125 + 225 ⁺ + 419

^a +, base peak.

^b Pentachlorobenzene.

^c Hexachlorobenzene.

^d Pentachloronitrobenzene.

ther comparison of results, a method of standard additions for GC/ITMS also was used to determine average pesticide concentrations in extracts from 8 potato subsamples.

(c) *Confirmation of pesticides.*—With GC/ITMS, the following criteria had to be met to confirm a pesticide in the sample: t_r difference of less than 10 s, signal-to-noise ratio greater than 3, and mass spectrum match greater than 90% versus the spectrum library for the pesticide (generated from pesticide standards). Only results for confirmed pesticides are presented in this paper.

Results and Discussion

Analysis

The SFE conditions and the GC/ITMS analysis used were similar to those in a previous study (7). Figure 1 shows a typical GC-ECD chromatogram (from Experiment 1) of a potato SFE extract containing PCB at 0.14 $\mu\text{g/g}$, HCB at 0.26 $\mu\text{g/g}$, lindane at 0.37 $\mu\text{g/g}$, and aldrin at 0.36 $\mu\text{g/g}$. As the figure shows, matrix interferences were negligible.

The extracts were analyzed by GC/ITMS. Table 2 lists the pesticides used, their retention times, and the quantitation masses. Figure 2 is a typical total-ion chromatogram of 40 pesticides fortified at 0.2 or 0.4 $\mu\text{g/g}$ in potato and extracted by SFE (Experiment 4A). Detection limits for pesticides analyzed by these methods were reported previously (5, 7).

Experiments 1 and 2

Table 3 summarizes the results of Experiments 1 and 2. The experiments were done to determine whether a small (1–2 g) subsample accurately represents a large (2.3 kg) sample from which it was taken. After the 2.3 kg sample was chopped with the Hobart cutter, recoveries of HCB and lindane from 48 replicate extractions of 2.1 g subsamples had relative standard deviations of 11–14%. Recoveries from 4 replicate subsamples from each 50 g portion had RSDs of 10–13%. The results shown in Table 1 for HCB and lindane were obtained with GC-ECD. The same samples were analyzed also by GC/ITMS, and reproducibilities were similar (RSDs: 10.3% for HCB and 12.6% for lindane). Recoveries of chlorpropham, an incurred residue in potato that was analyzed by GC/ITMS, had an RSD of 11.4%. The calculated concentration of chlorpropham, 4.2 $\mu\text{g/g}$, was corroborated by GC-NPD.

In Experiments 1 and 2, the amounts of HCB and lindane recovered from fortified potato were 63 and 85%, respectively. The losses, demonstrated by Experiments 3 and 4A, were due to evaporation during spiking, which was done at room temperature and in open air. When samples were frozen, losses were prevented, and recoveries became consistent.

In Experiment 2, a 50 g portion of potato from Experiment 1 was blended further with 100 g HMX. HMX enabled blending of the moist sample without use of a liquid, and the amount of HMX was adjusted to form a flowable material for proper mixing. The precision was greatly improved in Experiment 2, and

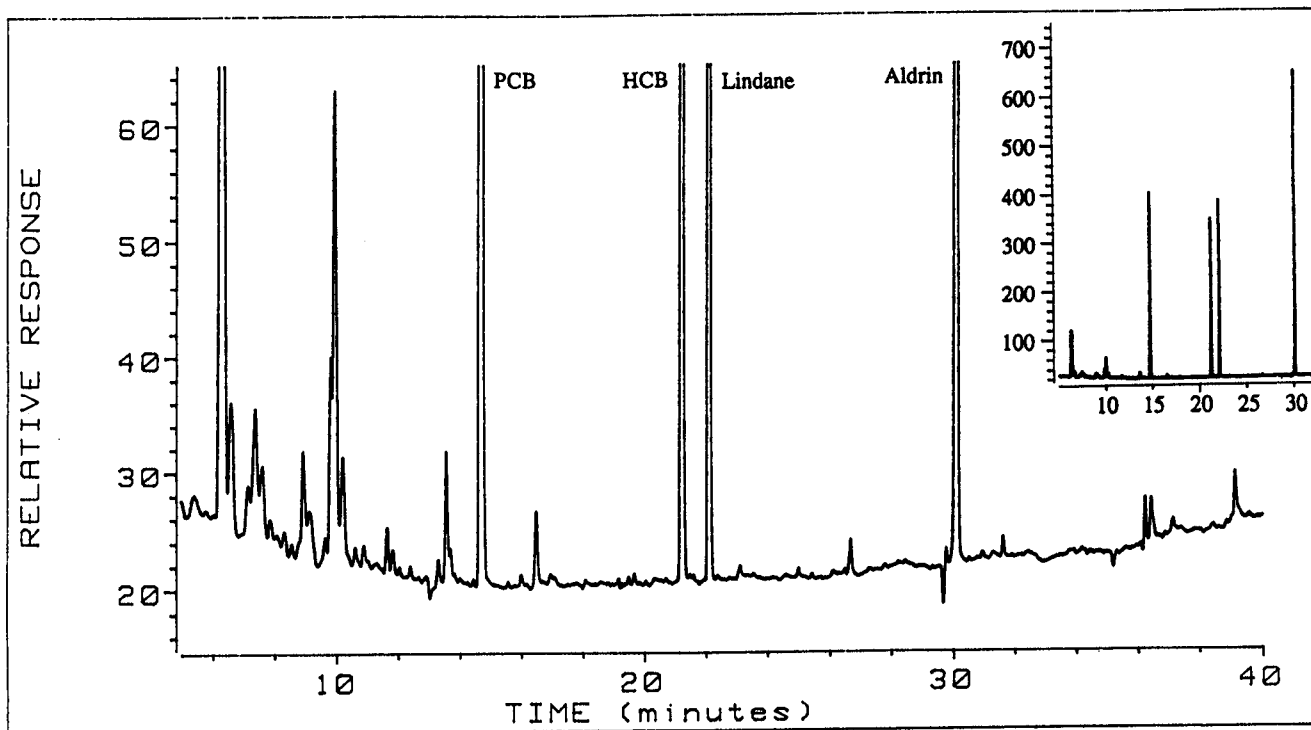


Figure 1. Typical GC-ECD chromatogram of potato extracted by SFE (Experiment 1). The full-scale chromatogram in the upper right shows the peak signals, and the larger chromatogram gives the noise levels of the potato extract, which was analyzed without additional cleanup. PCB, 0.14 $\mu\text{g/g}$; HCB, 0.26 $\mu\text{g/g}$; lindane, 0.37 $\mu\text{g/g}$; aldrin internal standard, 0.36 $\mu\text{g/g}$.

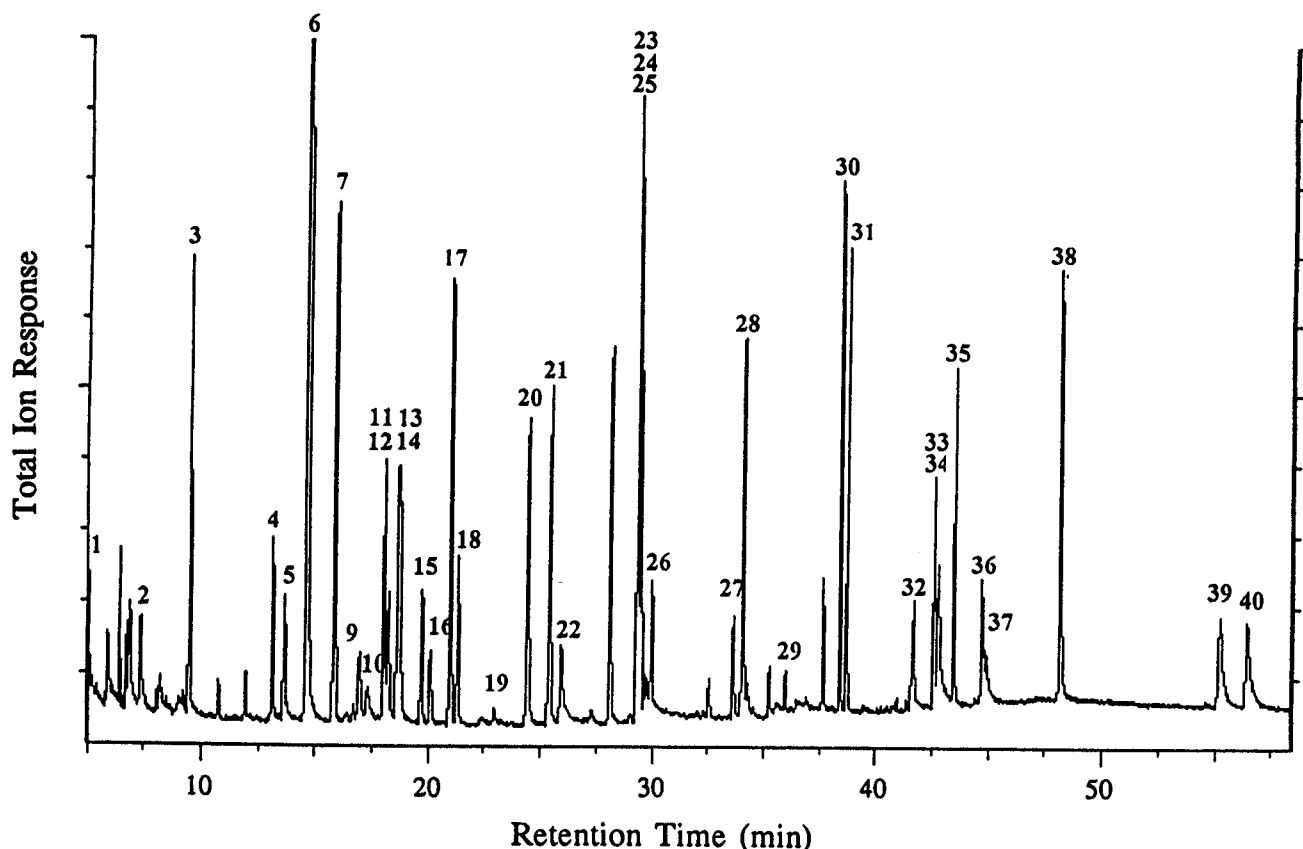


Figure 2. Typical GC/ITMS total-ion chromatogram of 40 pesticides fortified at 0.2 or 0.4 $\mu\text{g/g}$ in potato and extracted by SFE (Experiment 4A). The numbered peaks refer to the pesticides listed in Table 2.

recoveries were the same as those in Experiment 1. GC-ECD gave RSDs of 2.8% for HCB and 2.9% for lindane, compared with 10.9 and 13.6% respectively, in Experiment 1. GC/ITMS analysis gave RSDs of 2.6% for both HCB and lindane. For incurred chlorpropham, GC/ITMS analysis gave an RSD of 2.1%.

This excellent precision was obtained from as little as 1.3 g potato. Thus, pesticide residues in a 50 g sample, the amount commonly used in a current solvent-based method (20), were accurately determined with a 1.3 g subsample using SFE. Furthermore, the improvement in precision from an RSD of 10–14% in Experiment 1, in which 2.1 g subsamples were indi-

vidually chopped without HMX, to an RSD of 2–3% in Experiment 2, in which subsamples were blended with HMX, showed that greater sample homogeneity is achieved when samples are blended with HMX. Blending also may have created smaller sample particles, which are known to improve extraction by SFE (1–4).

Experiments 3 and 4

After Experiment 2, a similar experiment was carried out in which potato was spiked with a mixture of 40 pesticides. The blending procedure for 50 g potato was the same as in Experi-

Table 3. Reproducibility of repeated pesticide residue analyses of potatoes extracted by SFE (Experiments 1 and 2)

Pesticide	Overall sample size	Amount extracted by SFE, g	Number of replicates	Chopped without HMX		Blended with HMX	
				Conc., $\mu\text{g/g}$	RSD, %	Conc., $\mu\text{g/g}$	RSD, %
HCB	2.3 kg	2.1	48	0.26 ^a	10.9 ^a	—	—
HCB	50 g	1.3	9	—	—	0.28 ^a	2.8 ^a
Lindane	2.3 kg	2.1	48	0.37 ^a	13.6 ^a	—	—
Lindane	50 g	1.3	9	—	—	0.36 ^a	2.9 ^a
Chlorpropham	2.3 kg	2.1	48	4.21 ^b	11.4 ^b	—	—
Chlorpropham	50 g	1.3	9	—	—	4.49 ^c	2.1 ^b

^a GC-ECD results. Analysis by GC/ITMS resulted in similar RSDs for samples prepared without and with HMX: 10.3 and 2.6 for HCB and 12.6 and 2.6 for lindane, respectively.

^b GC/ITMS result.

^c GC-NPD result.

Table 4. Effect of sample preparation procedures for SFE on recovery of 40 pesticides fortified in potato (Experiments 3 and 4A)

Pesticide ^a	Fortification in vessel (5 replicates)		Blended at room temp. (Experiment 3, 8 replicates)		Blended with dry ice (Experiment 4A, 5 replicates)	
	Recovery, %	RSD, %	Recovery, %	RSD, %	Recovery, %	RSD, %
Category 1						
Atrazine	96	11	96	8.1	95	4.2
Carbaryl	94	24	69	13	116	3.4
Carbofuran	98	5.1	96	12.1	104	6.7
Chlorpyrifos	100	2.0	91	8.3	91	2.0
Dacthal	101	2.9	73	6.4	105	2.1
DDE	99	1.7	97	19	94	6.2
DDT	94	2.4	78	8.7	94	1.4
Diazinon	98	2.7	92	9.5	96	2.1
Dicloran	95	7.8	86	9.5	94	4.4
Dimethoate	98	14	77	17	113	5.0
Endosulfan I	100	1.3	95	10	95	2.0
Ethion	88	7.8	70	9.3	89	1.3
Ethoprop	92	4.6	85	3.6	103	3.8
Lindane	104	1.9	88	5.5	103	1.8
Methidathion	86	9.1	70	11	101	1.7
Methoxychlor	91	3.9	80	8.2	98	3.5
Parathion	81	19	72	7.5	83	9.1
Parathion-methyl	76	6.7	68	9.8	89	4.3
PCNB	95	1.6	63	6.8	81	3.4
cis-Permethrin	95	3.6	62	18	87	0.8
Vinclozolin	99	6.4	85	5.1	97	1.6
Category 2						
Chlorothalonil	83	24	8	20	91	6.2
Dichlorvos	82	8.8	8	42	74	5.1
Diphenylamine	96	3.7	17	9.2	9	4.5
Disulfoton	93	4.9	20	29	36	3.2
HCB	99	2.9	56	8.7	90	4.3
Malathion	83	14	35	17	75	2.0
α -Mevinphos	68	24	52	11	104	1.7
PCB	97	3.9	13	41	89	7.1
Phorate	95	3.6	47	10	69	2.3
Phosalone	81	14	45	51	96	2.9
Phosmet	74	17	53	16	108	3.3
Phosphamidon	72	14	44	19	107	10
Terbufos	88	2.9	57	7.6	78	3.1
Not categorized						
Azinphos-methyl	74	33	ND ^b	ND	107	5.1
Chlorpropham	Incurred	24	Incurred	8.4	Incurred	7.7
Esfenvalerate	80	12	ND	ND	90	3.8
Fenvalerate	91	14	ND	ND	90	3.5
Iprodione	81	26	ND	ND	102	2.3
Propargite	85	8.0	ND	ND	83	4.7

^a PCNB, pentachloronitrobenzene; HCB, hexachlorobenzene; PCB, pentachlorobenzene.^b ND, not detected because of GC problems with pesticide.

Table 5. Losses of pesticides from fortified potato kept at room temperature for 5 h

Pesticide ^a	Recovery, %	
	Exposed to open environment	Stored in extraction vessel
HCB	55	95
PCNB	62	95
PCB	12	85
Phorate	48	72
Malathion	35	65
Chlorothalonil	8	52
Disulfoton	20	30
Dichlorvos	8	28

^a HCB, hexachlorobenzene; PCNB, pentachloronitrobenzene; PCB, pentachlorobenzene.

ment 2; results are presented in Table 4. The first 2 columns show recovery of pesticides added directly to an extraction vessel packed with blank potato sample. This served as a control spike to determine the efficiency of SFE unrelated to sampling aspects. Even though calibration curves were prepared in blank potato extracts, which reduce matrix effects, accurate quantitation by capillary GC of a few pesticides (mainly organophosphorus insecticides, such as phosphamidon, azinphos-methyl, and parathion-methyl) still posed some difficulties.

The next 2 columns in Table 4 present results from Experiment 3, in which potatoes were spiked with the pesticides in open air and then allowed to stand for 1 h at room temperature before blending with HMX. Pesticides listed in Category 1, were relatively stable and nonvolatile, and consistently gave acceptable recoveries, although some losses occurred. Category 2 pesticides gave higher losses due to evaporation and/or degradation during sample preparation. Five of the 6 pesticides listed as "not categorized" were not detected in Experiment 3 because of a GC problem and could not be classified in Category 1 or 2. Recovery of chlorpropham was not determined because it was incurred.

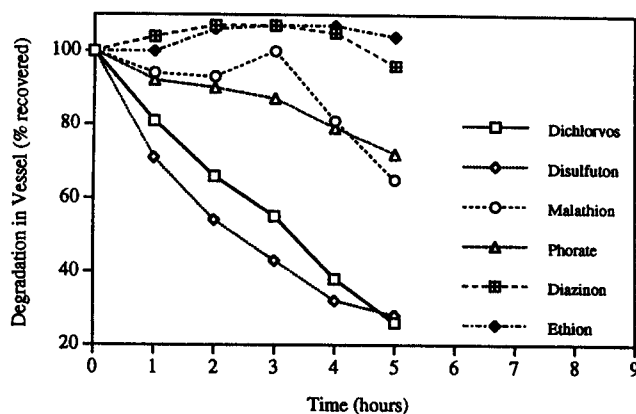


Figure 3. Degradation rates of 6 organophosphorus insecticides in potato samples kept in SFE vessels for 1–5 h at room temperature and exposed to air.

The final 2 columns present results of Experiment 4A, in which samples were kept cold with dry ice throughout sample preparation prior to SFE. Recoveries for most pesticides were 90–105%. Recoveries of many organophosphorus insecticides were more variable (75–116%) because of matrix effects when analyzed by capillary GC. Recoveries of repeated injections of these pesticides as standards in potato matrix solution fluctuated similarly. For most pesticides, recoveries in Experiment 4A were much better than those in Experiment 3, in which samples were not kept frozen throughout sample preparation.

The improved recoveries in Experiment 4A were also reflected in better precision. In Experiment 4A, RSDs varied from 0.8 to 10 % and typically were 3–4%, whereas in Experiment 3, RSDs fluctuated from 6 to 41%, mainly because of degradation or evaporation. Diphenylamine was the only pesticide tested that had lower recovery with addition of dry ice to potato. The recovery of disulfoton was slightly better but was still low (36%). Recoveries of other pesticides in Category 2, when compared with those in Experiment 3, were greatly improved.

Evaporation or Degradation

The experiments also provided data on losses of pesticides from the SFE vessel or during sample preparation in open air. Pesticides that were lost through volatilization could be separated from those lost through degradation. As shown in Table 5, the chlorinated pesticides PCB, HCB, and PCNB were lost only from samples exposed to air at room temperature. Once the samples were stored for the same period (5 h) in enclosed extraction vessels, losses were minimal. On the other hand, losses of pesticides such as dichlorvos, disulfoton, and malathion were due mainly to degradation. Degradation of these pesticides, as presented in Table 4 (Experiment 4A), was minimized by keeping samples frozen until extraction.

The results of Experiment 4B (Figure 3) show that degradation occurs in the extraction vessel over a period of several hours at room temperature as the vessels are awaiting extraction in sequence on the sample carousel. The organophosphorus insecticides, dichlorvos and disulfoton, were especially sensitive to degradation, with losses amounting to 70% of the original amount over a period of 5 h. Other pesticides like malathion and phorate degraded more slowly, whereas diazinon, ethion, and several others were not degraded. Except for disulfoton, the pesticides studied were kept from degrading by freezing the samples until they were extracted.

Pesticides in a sample kept for 13 h in the SFE chamber with CO₂ did not show any degradation. In a control sample, which was kept with air in the vessel for a similar period, some pesticides degraded completely. If a sequential automated sampler is being used for SFE, samples should either be kept frozen until extraction or stored in the vessel in the absence of oxygen.

Check Samples

The final sample preparation procedure was tested on peach and orange check samples. Table 6 compares results of the analyses with actual fortification levels and concentrations de-

Table 6. Analysis of peach and orange check samples

Sample	Pesticide	Actual conc., $\mu\text{g/g}$	Concentration detected, $\mu\text{g/g}$	
			SFE method	Traditional methods ^a
Peach	Dichlorvos	0.11	0.11 ± 0.021	0.10 ± 0.013
	α -Mevinphos	0.27^b	0.26 ± 0.043	0.29 ± 0.091^b
	Dicloran	Incurred	0.14 ± 0.013	0.12 ± 0.015
	Vinclozolin	0.095	0.098 ± 0.010	0.089 ± 0.014
	Iprodione	Incurred	0.68 ± 0.092	0.50 ± 0.12
Orange	Carbofuran	0.36	0.34 ± 0.013	0.31 ± 0.054
	Methoxychlor	0.19	0.14 ± 0.006	0.19 ± 0.048

^a Traditional analyses (19, 20) were performed by 7 laboratories participating in the Pesticide Data Program.

^b Fortification level was $0.39 \mu\text{g}$ of 70% α -mevinphos and 30% β -mevinphos per gram. Samples were analyzed for α -mevinphos only with the SFE method, and β -mevinphos results are not presented for the traditional methods.

terminated by 7 laboratories participating in the Pesticide Data Program (22) using traditional methods (19, 20). Figure 4 presents the calibration curves for the method of standard additions for 3 of the 7 pesticides. The results compare well in all cases, except for methoxychlor in the orange check sample. This low result is puzzling, because recovery of methoxychlor from fortified samples was $>90\%$. Recoveries of duplicate $0.5 \mu\text{g/g}$ fortification spikes in the vessels were 86% (RSD, 4%) for dichlorvos, 105% (RSD, 2%) for α -mevinphos, 105% (RSD, 3%) for dicloran, 105% (RSD, 4%) for vinclozolin, 117% (RSD, 0.5%) for iprodione, 128% (RSD, 1%) for carbofuran, and 94% (RSD, 1%) for methoxychlor. The check sample results further demonstrated the viability of the method for multiresidue analysis of pesticides in fruits and vegetables. The correct determination of dichlorvos in the peach sample showed that the sample preparation technique adequately controls losses of a volatile and unstable pesticide in the sample.

Conclusions

The goal of this study was to develop practical procedures for preparing fruit and vegetable samples for extraction by SFE in for multiresidue analysis of pesticides. Traditionally, an organic solvent distributes the analyte in a solution and helps to avoid losses from volatilization and degradation. With SFE, the amount of sample required is small (1–3 g) and the analyte is distributed in a moist solid matrix. Sample homogeneity was accomplished by blending the sample with HMX to form a flowable powder. A 2:1 HMX:sample ratio successfully reduced moisture to the point that the sample did not stick to itself or to the blender wall. With dry ice, a 1:1 HMX:sample ratio gave the same effect, thus increasing the amount of sample packed into a fixed-volume extraction vessel.

If a large (2.3 kg) frozen sample was chopped only with a Hobart cutter, without blending with HMX, results from ex-

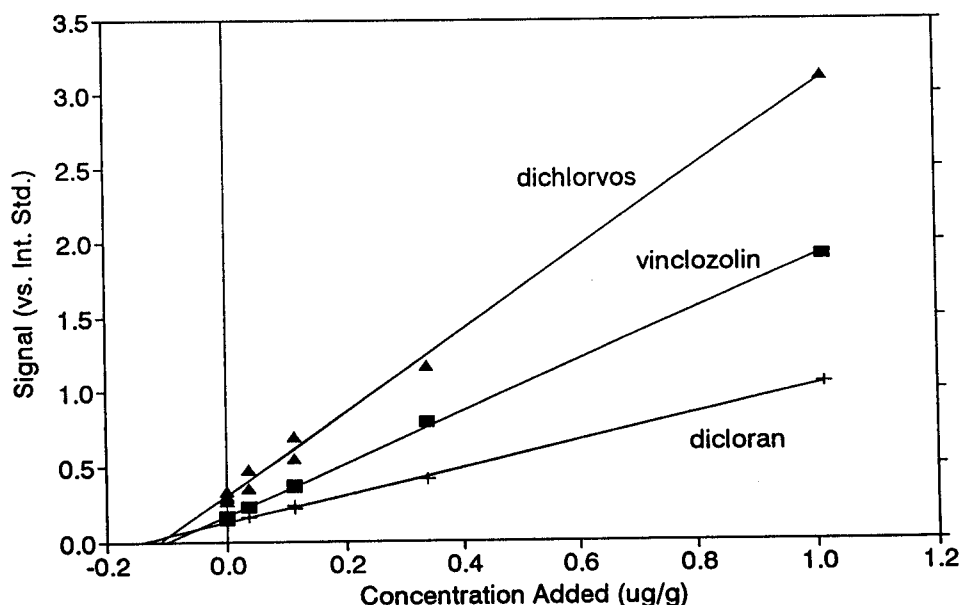


Figure 4. Determination of dichlorvos, vinclozolin, and dicloran in peach check sample using the method of standard additions.

tractions of 2.1 g subsamples gave an RSD of 10–14%. By adding HMX and forming a flowable material of a 50 g sample in a blender, the precision of the extraction of even smaller subsample amounts (1.3 g) was greatly improved (RSD, 1–6%). In most cases, high variations were related either to pesticide stability in the solid matrix or to capillary GC analysis and not to extraction efficiency. The precision of recovery results was usually limited by the reproducibility of the analytical method and not by sample preparation or SFE.

Some pesticides rapidly degraded and/or evaporated when exposed to air at room temperature. Volatilization was practically eliminated by keeping the sample frozen during sample preparation and by reducing sample exposure to an open environment. Once the sample was packed into an extraction vessel, evaporation was minimal. However, degradation of certain pesticides still occurred in the vessel as long as O₂ was present. When the sample vessels were loaded into the autosampling carousel for sequential extraction, several pesticides, especially some of the organophosphorus insecticides, degraded over a period of hours. By purging a packed vessel with gases such as N₂ or CO₂, oxidative degradation can be avoided, as shown by analysis of dichlorvos and α -mevinphos in the peach check sample and results for other pesticides in check samples being comparable with results from traditional analyses. The procedures offer a practical approach for sample preparation for SFE of plant materials for multiresidue analysis of pesticides.

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